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PHARMACOLOGICAL STUDIES ON THE ANTITUSSIVE, EXPECTORANT AND ANTIASTHMATIC EFFECT OF LUTEOLIN-AN ACTIVE PRINCIPLE OF AJUGA DECUMBENTS THUMB

[Baimao Xiakucao De Youxiao Chengfen—Muxicaosu Dui Ke-Tan-Chuan Yaoli Zuoyong De Yanjiu]

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PHARMACOLOGICAL STUDIES ON THE ANTITUSSIVE, EXPECTORANT AND ANTIASTHMATIC EFFECT OF LUTEOLIN-AN ACTIVE PRINCIPLE OF AJUGA DECUMBENTS THUMB

Ajuga decumbens Thunb., which is also called *Ajuga ciliata* Bunge, is a Labiatae plant that can be fully used as herbal medicine, Its acid alcohol extract has been made into sugarcoated tablets and used for clinical treatment of chronic bronchitis[1]. The herb is proved to be effective to varying degrees on the treatment of tussis, expectorant, asthma, and inflammation. It is especially effective on the treatment of tussis and expectorant. It is verified that the active principle separated from the herb is *Luteolin*. The pharmacological and toxicological studies on the effect of *Luteolin* are briefly described as follows:

Antitussive Effect Experiment

Experimental method: Apply constant-pressure concentrated ammonia spray (25~28% NH_4OH) for the duration (ET_{50}) necessary to cause half of the mice in test to cough[2]. The experiment adopts an "up-down" method, i.e., each group has 10 mice and the duration (ET_{50}) necessary to cause half of the mice in the

[1] Numbers in the margin indicate pagination in the foreign text.

control group to cough is marked as 100%. The antitussive effect is considered as "effective" and "significantly effective" when the ET_{50} used in the test group is 130% and 150% more than that in the control group, respectively.

Experimental results: See attachment table.

Attachment Table

Application Method	Dosage (mg/kg)	$\frac{\text{Test Group } ET_{50}}{\text{Control Group } ET_{50}} \times 100\%$	Effect Evaluation
Gastric perfusion	250	175.0	Significantly effective
Abdominal administration	125	156.6	Significantly effective

It can be seen from the table that *Luteolin* is proved to have strong and stable antitussive effect through regardless of gastric perfusion or abdominal administration.

A. Effect of *Luteolin* on cat tussis caused by electric stimulation of superior laryngeal nerves of anaesthetized cat

Experimental method: Inject 3% pentobarbital sodium anesthetic into the abdominal cavity of a healthy cat. Follow the Domehjoz et al.[3,4] method to segregate superior laryngeal nerves. Put a segment of nerves on a platinum conductor for stimulation. Follow the Toner method[5] to coat some low melting point wax on the electrode and nerve surface to prevent short-

circuit caused by leaking liquid. In order to record tussis reaction, a self-made glass hose is inserted into the cat's oral cavity. The outlet end of the hose is connected to an aerotympanum via a T-duct to record the cat's normal respiration and tussis reaction. Use Heinz Netheler electronic stimulator with the following parameters: stimulation frequency: 10~65 cycles/s, current intensity: 0.3~2mA, pulse time: 0.1~0.2ms, stimulation time: 4~5s. Once a proper stimulation threshold is determined, the cat makes more than one time tussis and noise under the continuous stimulation of 3~5s. When 3~5 times tussis reaction becomes stable, the medicine is injected into the cat's femoral vein or abdominal cavity. Measure the tussis reaction based on the original threshold value in 5, 15, 30, 60 and 120 minutes after injection. If the tussis intensity or frequency is suppressed after injection, the medicine is considered to have antitussive effect.

Experimental results: 19 cats were used in the experiments and *Luteolin* was applied 19 times. In 5~15 minutes after vein injection of 30mg/kg *Luteolin*, the tussis reaction was completely suppressed, indicating that *Leteolin* has significant antitussive effect and the effect can last for 2 hours. The experiment shows that *Leteolin* acts on the cat's nerve center instead of reducing the sensitivity of receptors in the trachea.

B. Effect of *Luteolin* on cat tussis caused by electric stimulation of superior laryngeal nerves of cerebrum-removed cat

Experimental method: Remove the cerebrum of a healthy cat under ether anesthesia[6] and keep the brain stem below quadrigeminal bodies. Segregate the superior laryngeal nerves for antitussive experiment. The measurement method and conditions are basically the same as those described above./12

Experimental results: 4 cats were used in the experiment. The result shows that the antitussive effect exists in either vein injection or abdominal administration after the cerebrum hemispheres on both sides are removed. This further proves that the antitussive effect of *Luteolin* is not on the cerebrum but on the brain stem.

C. Effect of *Luteolin* on tussive center of brain stem

Experimental method: Follow the Chakravarly method[7] to laterally remove the cat's cerebrum at the middle dorsal thalamus position under ether anesthesia. Drill open the occiput and skull, and use a medical spoon to carefully pick out lumbrical part of the cerebellum so as to expose the rhomboid fossa on the backside area of the medulla. Promptly segregate the trachea and insert a T-duct into the trachea. The side duct is connected to an aerotympania in order to record tussis reaction. After operation, lightly insert a dual-pole needle electrode into the tussis center in the backside area of the

medulla with the assistance of a stereo orientation device. Connect the electrode to the output of the electronic stimulator. The stimulation parameters are as follows: frequency: 55 cycles/s, pulse time: 2ms, intensity: 2mA. Continuous tussis reaction is induced in 5 seconds after stimulation. Stimulation is applied once every 5 minutes. After 3 consecutive, stable tussis reactions are obtained, inject 60mg/kg *Luteolin* and measure the tussis reaction based on the original threshold value in 15, 30, and 60 minutes after injection.

Experimental results: 3 cats were used in the experiment and *Luteolin* was applied 3 times. As shown in the attachment diagram, no tussis reaction took place under stimulation with original parameters in 15, 30, and 60 minutes after injection of *Luteolin*.

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1 Before injection

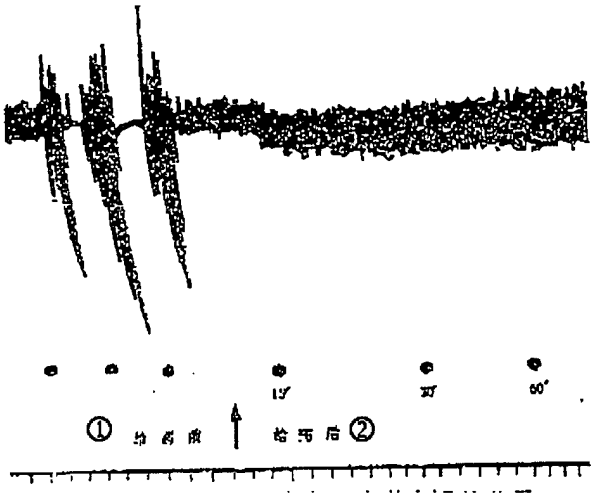
2 After injection

3 Cat ♂ 2.65kg

4 • Stimulation mark

5 Time scale: 30s per division

6 T *Luteolin*



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Attachment Diagram: Effect of *Luteolin* on Brain Stem Tussis Center

The attachment diagram shows that *Luteolin* acts directly on the tussis center of the brain stem.

Expectorant Effect Experiment

A. Small mice phenol red method[2]: *Luteolin* is considered to have expectorant effect when the phenol red concentration in the test group is 200% more than that in the control group and when difference of statistical results between the two groups is significant. Experimental results: Under 100mg/kg gastric perfusion, the phenol red concentration in the test group is 1.00μg/ml, which is 370% that of the control group ($P<0.05$), indicating that *Luteolin* has good expectorant effect.

B. Big mice capillary drainage method[8]: *Luteolin* is considered to have expectorant effect when the average secretion amount per hour before administration is in normal range and the

average secretion amount per hour after administration is 170% of the normal value. The experimental results show that the secretion in the respiratory tract of big mice increased significantly after gastric perfusion of 20mg/100g *Luteolin*. The average secretion per hour after administration is about 550% of the normal value, indicating that *Luteolin* has good expectorant effect.

Studies on Expectorant Effect

A. Effect of gastric perfusion after cutting off vagus nerve and abdominal administration after cutting vagus nerve on the secretion of respiratory tract

The experimental results show that *Luteolin* has clearly expectorant effect after the vagus nerve of the mouse is cut off in either gastric perfusion or abdominal administration conditions, and that the secretion in the trachea is not affected by the cutoff of vagus nerve. This indicates that the expectorant effect of *Luteolin* is not induced through stimulation of reflection of chemical receptors in the gastric-duodenum mucosa.

B. Effect of *Luteolin* on the dissolution of acid mucopolysaccharide fibers in expectoration

It is discovered through microscope observation that depolymeration in varying degrees took place in acid mucopolysaccharide fibers in expectoration after treatment with

Luteolin, indicating that the expectorant effect of *Luteolin* has something to do with the dissolution of mucopolysaccharide fibers in expectoration.

Effect of *Luteolin* on Isolated Guinea Pig Trachea

Experimental method[9]: Strike guinea pig to faint and cut the artery to make the guinea pig bleed to death. Incise the jugular skin to peel off a segment of trachea. Fix one end of the trachea on a J-shaped metal tube and the other end on an L-shaped glass tube of about 1mm inner diameter. Place the vertical parts of the J-shaped tube and L-shape glass tube for fixing the trachea in a constant-temperature bath. Use a small air-blowing device to continuously blow air into the bath. Add 0.1ml 1% acetylcholine and 0.1% histamine mixed solution (1:1). Because of trachea contraction, the solution in the glass tube moves fast towards the tube end. Note down the mm value of solution forward movement to indicate the intensity trachea contraction caused by histamine and acetylcholine. After that, add 0.1ml (about 1mg) *Luteolin* and note down the mm value of solution backward movement in 10 minutes after *Luteolin* addition. Determine the percentage of *Luteolin* effect against acetylcholine and histamine as indication of the strength of its trachea expansion effect. /13

Experimental results: The effect of 1mg *Luteolin* against contraction agent is about 100% or over, indicating that

Luteolin has direct expansion effect on the smooth muscle if isolated guinea pig trachea.

Effect of *Luteolin* on the Strip Muscle of Isolated Guinea Pig Spiral Trachea

Experimental method[10]: Strike a guinea pig to death and immediately peel off one segment of its trachea. Use iris scissors to cut the trachea, in 45° inclination from thyroid cartilage, downward along the spiral line into a 2~3mm wide spiral trachea strip. The lower end of the trachea strip is fixed on a metal hook at the bottom of the bath while the upper end is connected to a universal lever. Add 2ml 1% acetylcholine and 0.1% histamine mixed solution (2:1) and observe the effect of *Luteolin* on the strip muscle of the trachea under 0.5, 1, and 2mg dosages.

Experimental results: 5 samples were used in the experiment and *Luteolin* was applied 6 times. The experimental results show that *Luteolin* can antagonize the excitation effect of histamine and acetylcholine on the trachea strip muscle and can thus release the trachea's contracture. The antagonism becomes remarkable when the dosage gradually increases.

Effect of *Luteolin* on Muscle Tonicity of Guinea Pig Bronchus

Experimental method[11, 12]: Anaesthetize a guinea pig and fix it on the back position. Insert a trachea cannula and connect it to a self-made bronchus muscle tonicity sensing device. Apply

cavity injection of 100mg/kg erythromycin to the mice in the erythromycin group. Within three days, the death rate in the control group is 100%, compared to 50% in the *Luteolin* group and 60% in the erythromycin group. This indicates that *Luteolin* also has high internal anti-infection effect.

Acute Toxicity Test

No mouse is seen poisoned to death at maximum oral dosage of 2500mg/kg *Luteolin*. The LD_{50} under abdominal injection is 180mg/kg.

Subacute Toxicity Test

Apply gastric perfusion of *Luteolin* to guinea pig every day (the dosage is equivalent to 50 times that of adult daily dosage) for 20 days. No effect and changes are observed to the animal's general conditions, liver and kidney functions, electrocardiogram, peripheral hemogram, and other important and essential organs (heart, liver, spleen, lungs, kidney, stomach, thyroid gland, and suprarenal gland). It proves that the toxicity of *Luteolin* is very little and implies that long-term dosage of *Luteolin* by clinical patients is safe.

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Luteolin from jugular vein and note down the change of tonicity of the bronchus smooth muscle.

Experimental results: 8 guinea pig were used in the experiment and *Luteolin* was applied 7 times. First apply intravenous injection of histamine at 50μg/guinea pig. The contraction of the bronchus smooth muscle caused the curve amplitude to increase. Following vein injection of 10mg/100g *Luteolin*, the curve lowered clearly in 3~5 minutes after administration. The curve level was about 1/2 lower than that of normal curve and effect lasted for 30~40 minutes. The experimental results show that under nearly normal innervation and blood supply of animal bronchus and lungs as a whole, *Luteolin* can relax animal's bronchus and microbronchus smooth muscles.

Bacteriostasis of *Luteolin*

The external bacteriostasis experimental results show that *Luteolin* (10mg/ml) has comparatively stable bacteriostasis against *Staphylococcus aureus*, alpha streptococcus, catarrhcoccus, pneumococcus, and *Pseudomonas aeruginosa*.

Internal anti-infection experiment[13]: Pick 30 mice and divide them into three groups. Inject *Staphylococcus aureus* broth bouillon into abdominal cavities of the mice. Apply gastric perfusion of 700mg/kg *Luteolin* twice before and after infection to the mice in the *Luteolin* group. Apply abdominal

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